



Morphometrical Analysis of the Reproductive System in Boschveld Chickens

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ABSTRACT

The egg yield is determined by the number of ovarian follicles that ovulate and the oviduct's capability to form a hard-shelled egg around the egg cell. However, the morphological characteristics of the reproductive organs in Boschveld chickens have not been explored. The present study aimed to evaluate the morphology of the reproductive system, including the oviduct and the developing ova in the Boschveld chicken breed. To perform a morphological analysis, ovarian follicles and oviducts were obtained from six Boschveld hens (Out of a total of twenty), aged 30 weeks, sourced from the University of Limpopo experimental farm in South Africa. The present results revealed significant differences in both the weight and number of small white (2-4 mm), large white (5-6 mm), and small yellow (7-8 mm) follicles. Among the mature follicles, Follicle 1 (F1) exhibited the highest weight, whereas F6 demonstrated the lowest weight. Additionally, the weights and lengths of the infundibulum, magnum, isthmus, and shell gland varied significantly in Boschveld chickens. The present findings indicated that the magnum was the heaviest and longest oviduct segment in Boschveld chickens, and the heaviest pre-ovulatory follicle was ovarian F1, followed by ovarian F2. The results revealed a distinct profile characterized by many small white follicles and a well-developed magnum, which is the largest part of the oviduct. These findings provided a crucial anatomical baseline for the breed, indicating that the structure of the follicular hierarchy and oviduct plays a crucial role in its egg-laying ability. Improving the development and function of these reproductive parts could boost productivity in the Boschveld breed.

Keywords: Boschveld chicken, Isthmus, Magnum, Morphology, Ovarian follicle

INTRODUCTION

Poultry production is a vital part of the agricultural sector, significantly contributing to the global food system by supplying meat and eggs as primary protein sources for consumers (Vlaicu et al., 2024). The demand for poultry production has increased alongside the growth of the global population in KwaZulu-Natal, South Africa (Kleyn and Ciacciariello, 2021). The Boschveld chicken, a breed developed in South Africa, is well-known for its adaptability to free-range farming and its dual-purpose utility for both meat and egg production (Manyelo et al., 2020). Boschveld chicken is known for its robust characteristics, including disease resistance and strong brooding ability, making Boschveld hens well-suited to harsh environmental conditions (Hlokoe and Tyasi, 2022). Despite these advantages, the Boschveld breed has relatively low egg production, which limits its profitability in intensive or semi-intensive farming systems (Okoro et al., 2017). Consequently, if the sequential growth and development of ovarian follicles remain stagnant at the developmental stage, egg production in chickens will decline (Xu et al., 2024). The morphology and function of the chicken's reproductive system, particularly the oviduct and ovarian follicles, are directly related to egg productivity (Chen et al., 2021). Investigations into ovarian follicle development and oviduct characteristics in different breeds, such as Potchefstroom Koekoek and hybrids of White Leghorn, have highlighted significant morphological variations influencing egg-laying performance (Nie et al., 2022). Therefore, the present study aimed to determine the morphological variations of the ovarian follicles and identify the weights and lengths of the oviduct in Boschveld chickens.

MATERIALS AND METHODS

Ethical approval

The Animal Research Ethics Committee (AREC), certified by the National Health Research Ethics Council of South Africa, approved the present study (Registration Number: AREC-290914-017).

Study area

The current study was conducted at the Experimental Farm of the Limpopo University, situated approximately 10 km northwest of the Turfloop campus in South Africa. Summer temperatures (November to January) differs from 20°C to 36°C, whereas winter temperatures (May to July) ranged from 5°C to 25°C. The farm is positioned at a latitude of 27.55°S and a longitude of 24.77°E, with an average annual rainfall of less than 400 mm (Hlokoe *et al.*, 2023).

Study design

A total of 20 Boschveld hens, each weighing approximately 1.7 kg, were acquired at 20 weeks of age from Boschveld Farm, South Africa and subsequently reared at the Experimental Farm of the University of Limpopo, South Africa. The hens were housed in an intensive production system under consistent environmental conditions. The interior temperature of the poultry house ranged from 20 to 21°C, with relative humidity maintained between 50% and 60% to ensure the health and well-being of the poultry (Alabi *et al.*, 2012). The Boschveld hens were raised within an enclosed poultry house under a controlled production system. The side openings were fitted with PVC vinyl curtains to regulate ventilation, which were kept open during daylight hours and closed at night to safeguard the hens from rodents and predators. During the laying period, a lighting schedule of sixteen hours of light and eight hours of darkness was maintained in accordance with the recommendations of Nassar *et al.* (2017). One week before the arrival of the chickens, the poultry house was meticulously cleaned and disinfected utilizing Virokill disinfectants (Dalton, UK) at a concentration of 100 grams per 10 liters of water, to prevent the occurrence of diseases. Biosecurity protocols were stringently implemented, including the use of footbaths with Glutaraldehyde-based disinfectants (Deluxe Chemicals, South Africa) at the entrance to ensure appropriate sanitation prior to entry house, as described by Alabi *et al.* (2012). The hens were provided with laying mash (Driehoek Feeds, South Africa) starting at 20 weeks and had unrestricted access to water. The diet consisted of the following nutritional components, including crude protein (16%), crude fats and oils (4.3%), crude fibres (4.8%), crude ash (13.6%), calcium (4.3%), phosphorus (0.6%), sodium (0.15%), lysine (0.7%), methionine (0.35%) and metabolizable energy (2453.60 Kcal/kg). The diet was formulated in accordance with NRC (1994) standards. Chickens received vaccinations against Marek's disease (Prevexxion® RN vaccine, Boehringer Ingelheim Animal Health Ltd, South Africa) on the first day via subcutaneous injection, against Newcastle disease with B1 strain (LaSota) vaccine (BioMedical International Pvt Ltd, India) through oral administration on day one, and against Gumboro disease (Nobilis® Gumboro 228E vaccine, Merck Sharp and Dohme Animal Health, Netherlands) on day fourteen through drinking water following the procedure by Iqbal and Khan (2023). At thirty weeks of age, six laying hens were randomly selected and euthanized to facilitate the collection of ovarian follicles and oviducts for morphological analysis.

Collection of ovarian follicles and oviducts

The selected 30-week-old Boschveld hens were euthanized, following the method described by (Jacobs *et al.*, 2019). Before euthanizing, the hens underwent fasting for eight to twelve hours to ensure that their digestive tracts were emptied, as noted by Mfoundou *et al.* (2021). The chickens were euthanized by initially performing cervical dislocation, which is a method of animal euthanasia involving the rapid dislocation of the neck, leading to death through the disruption of the spinal cord and blood circulation to the brain (Jacobs *et al.*, 2019). The pharynx, neck arteries, cervical veins, oesophagus, and windpipe were then sliced without severing the head. The carcasses were depilated and dissected by cutting from the oesophagus down to the stomach, exposing the reproductive system to collect the oviduct and ovarian follicles, as described by Nassar *et al.* (2017). Moreover, the oviducts were collected following the method described by Mohammadpour *et al.* (2012). The ovarian follicles were collected from the reproductive system of the Boschveld hens by dissecting the hen into two halves. The ovarian follicles were then categorized according to their size (Table 1). The ovarian follicles were then categorized according to their size (Table 1). The largest pre-ovulatory follicles were hierarchically ranked and designated as Follicle 1 (F1) to F6. The weights of the small yellow follicle (SYF), large white follicle (LWF), and small white follicle (SWF) were measured using an electronic weighing scale (Medidata®, USA) with a precision of 0.01 g, while the lengths and widths of the SYF, LWF, and SWF were determined with vernier calipers (Mitutoyo® digital, Japan) with an accuracy of 0.01 mm. The number of large yellow follicles (LYF), SYF, LWF, and SWF were evaluated and recorded. The length of the ovarian follicles was measured with a vernier caliper. The total weight and length of the oviduct were measured with an electronic scale and vernier calipers, respectively. The oviducts from the six Boschveld carcasses were removed from their reproductive systems and dissected into four parts, including the infundibulum, isthmus, magnum, and shell glands. The weight of each oviduct section was recorded with an electronic scale, and the lengths and widths of the segments were measured with vernier calipers and a tape measure calibrated in centimeters.

Table 1. Follicle categories of 30-week-old Boschveld chickens

Follicle type	Size range (mm)
Small yellow follicle (SYF)	6 - 8
Large yellow follicles (LYF)	9 - 37
Follicle 1 (F1)	> 37
Follicle 2 (F2)	32 - 36
Follicle 3 (F3)	29 - 31
Follicle 4 (F4)	25 - 28
Follicle 5 (F5)	20 - 24
Follicle 6 (F6)	>20

Source: Wang et al. (2017). mm: Millimeters. The largest pre-ovulatory follicles were hierarchically ranked from F1 (largest) to F6 (smallest).

Statistical analysis

The current findings were analyzed using the Statistical Package for Social Sciences (SPSS) version 29.0 (IBM SPSS, 2023). Descriptive statistics, presented as Mean \pm Standard Error (SE), were used to summarize the data regarding the morphology of ovarian follicles and oviducts. A one-way Analysis of Variance (ANOVA) was applied to examine variations among ovarian pre-hierarchical follicles and between the morphologies of ovarian follicles and oviducts. Statistical significance was set at $p < 0.05$.

RESULTS

Weight of chickens, oviducts, and pre-hierarchical follicles

Table 2 presents the differences among ovarian pre-hierarchical follicles, including the weights of SWF, SYF, and LWF. The current findings indicated significant differences in the weights of pre-hierarchical follicles, specifically revealing that the weight of SWF was substantially more than that of SYF and LWF ($p < 0.05$). However, no significant difference was found between the weights of SYF and LWF ($p > 0.05$). The average weight of the chickens was 3.50 kg, while the average weight of the oviducts was 43.53 g.

Table 3 indicates the differences in the number of ovarian pre-hierarchical follicles of 30-week-old Boschveld chickens. The present results indicated a significant difference in the number of SWF being greater than that of LWF and SYF ($p < 0.05$).

Table 2. Weight of chickens, oviducts, and pre-hierarchical follicles of 30-week-old Boschveld chickens

Parameters	Mean \pm SE
Weight of small white follicles (SWF)	6.27 ^a \pm 0.24
Weight of small yellow follicles (SYF)	0.55 ^b \pm 0.08
Weight of large white follicles (LWF)	0.56 ^b \pm 0.09
Average weight of chickens	3.50 \pm 0.76
Weight of oviducts	43.53 \pm 0.12

SE: Standard error of means. ^{a, b, and c} Means with the different superscript letters in the same column are significantly different ($p < 0.05$).

Table 3. Number of pre-hierarchical ovarian follicles of the 30-week-old Boschveld chickens

Parameters	Mean \pm SE
Number of small white follicles (SWF)	53.83 ^a \pm 1.05
Number of large white follicles (LWF)	2.50 ^c \pm 0.22
Number of small yellow follicles (SYF)	4.50 ^b \pm 0.22

SE: Standard error of means. ^{a, b, and c} Means with the different superscript letters in the same column are significantly different ($p < 0.05$).

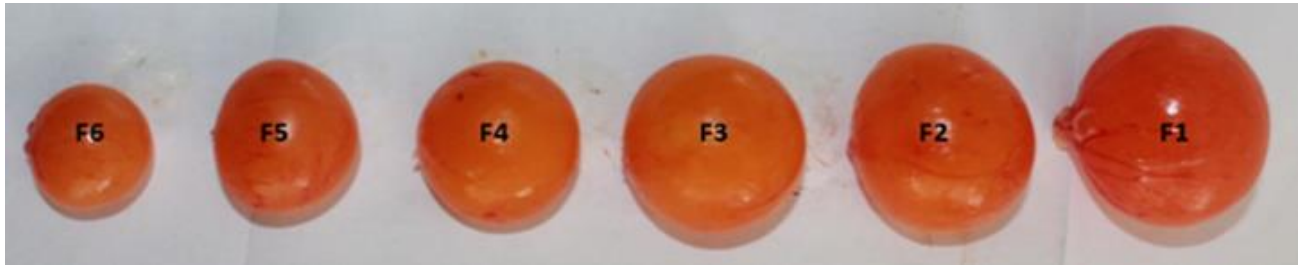
Number and weight of pre-ovulatory follicles

Table 4 illustrates the weights of pre-ovulatory follicles (F1 to F6). Significant differences were found among pre-ovulatory follicles, with F1 having the highest weight compared to other pre-ovulatory follicles ($p < 0.05$; Figure 1).

Table 4. Weights of pre-ovulatory follicles in 30-week-old Boschveld chickens

Parameters	Weight (Mean \pm SE)
Follicle 6 (F6)	12.51 ^f \pm 0.09
Follicle 5 (F5)	21.28 ^e \pm 0.09
Follicle 4 (F4)	27.42 ^d \pm 0.05
Follicle 3 (F3)	29.44 ^c \pm 0.12
Follicle 2 (F2)	35.44 ^b \pm 0.12
Follicle 1 (F1)	39.37 ^a \pm 0.09

SE: Standard error of means. ^{a, b, c, d, e, and f} Means with the different superscript letters in the same column are significantly different ($p < 0.05$).

**Figure 1.** Classification of pre-ovulatory follicles (F1- F6) of 30-week-old Boschveld chickens

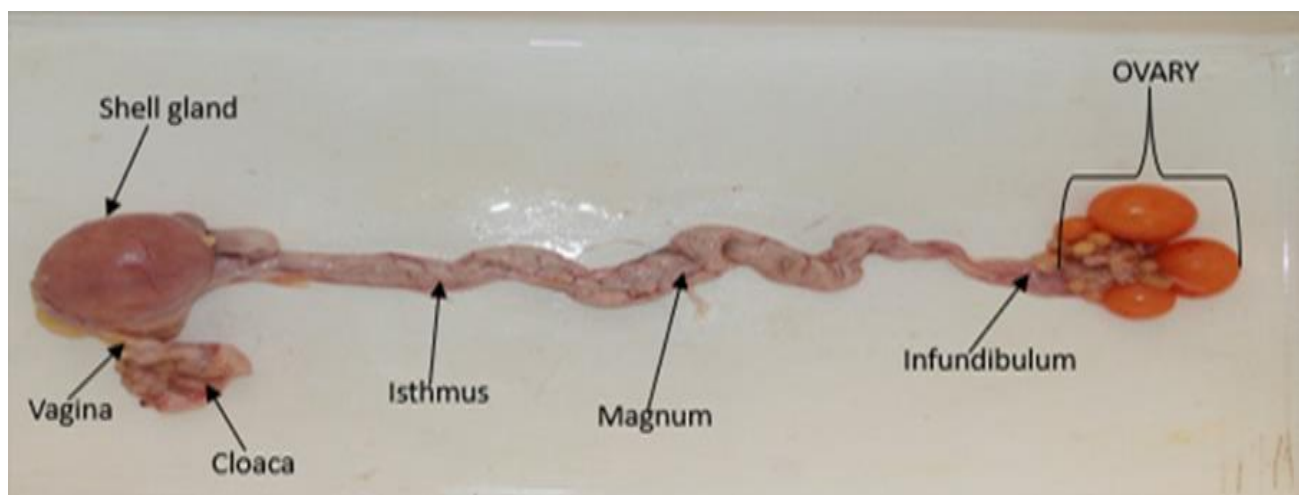
Weights and lengths of the oviduct segments

Table 5 presents the differences between the weights and lengths of oviduct segments. The weights of the infundibulum, magnum, isthmus, and shells gland were significantly different ($p < 0.05$). The magnum segment weighed more than the shell gland, isthmus, and infundibulum. The current findings exhibited that the lengths of the infundibulum, magnum, isthmus, and shell gland varied significantly ($p < 0.05$), with the magnum segment being larger than the infundibulum, isthmus, and shell gland. Figure 2 illustrates the different oviduct segments of the 30-week-old Boschveld chicken.

Table 5. Oviduct segments of 30-week-old Boschveld chickens

Parameters	Mean \pm SE
Weight of the infundibulum	9.70 ^c \pm 0.11
Weight of magnum	32.26 ^a \pm 0.12
Weight of the isthmus	3.52 ^d \pm 0.18
Weight of the shell gland	20.36 ^b \pm 0.16
P-value	$p < 0.05$
Length of the infundibulum	12.18 ^b \pm 0.96
Length of magnum	34.56 ^a \pm 0.08
Length of isthmus	7.30 ^d \pm 0.09
Length of the shell gland	9.39 ^c \pm 0.09
P-value	$p < 0.05$
Length of oviduct	63.42 \pm 0.12

SE: Standard error of means. ^{a, b, c, and d} Means with the different superscript letters in the same column are significantly different ($p > 0.05$).

**Figure 2.** Oviduct anatomy of the Boschveld chicken. Source of the image: captured by the authors of the present study.

DISCUSSION

The number of ovarian follicles designated for ovulation or atresia, along with the oviduct's capacity to effectively convert ova into a hard-shelled egg, serve as indicators of optimal productivity (Yang et al., 2019). The present study revealed significant variation in the weights of pre-hierarchical ovarian follicles, with SWF weighing more than SYF and LWF. The current findings illustrated that there was no significant difference between the weights of SYF and LWF. Moreover, the number of SWF exceeded those of both LWF and SYF. Additionally, significant differences were observed in the weights of pre-ovulatory follicles, with F1 being the heaviest among all pre-ovulatory follicles. The results of the current study contrast with the findings of Nie et al. (2022), who reported that, in yellow-bearded chickens, LYF were more numerous and heavier than both SYF and LWF. Such discrepancies could be attributed to differences in chicken breeds. Nassar et al. (2017) identified significant differences in the number of LYF and SYF in chickens of the Cairo L-2 strain and the LBL strain. The current findings implied that SWF may play a vital role in understanding the reproductive potential of the breed, as a higher number of SWF could indicate an increased capacity for ovulation. Wang et al. (2017) and Li et al. (2019) asserted that the different stages of ovarian follicle growth and development influence the efficiency of egg production in chickens.

In addition, the current study characterized the weights and lengths of oviduct segments in Boschveld chickens using descriptive statistics. The present findings indicated that there were significant differences in the weights of the infundibulum, magnum, isthmus, and shell gland segments. The weight of the magnum segment was higher than the weight of the infundibulum, isthmus, and shell gland. The lengths of the infundibulum, magnum, isthmus, and shell gland differed significantly in the current study. The magnum was longer than the infundibulum, isthmus, and shell gland segments. The current results are consistent with the findings of Mahajan and Joshi (2020) in White Leghorn chickens and those of Beulah et al. (2024) in Siruvidai chickens, who found that the magnum was the longest segment of the oviduct and the weight was higher than that of the infundibulum, isthmus, and shell gland. The results of Mahmud et al. (2017) on Nigerian local chickens indicated that the mean values and lengths of the infundibulum, magnum, and isthmus were statistically similar among the three studied genotypes. Kheawkanha et al. (2021), on the other hand, observed no statistically significant differences in oviductal weight and length among mature and old hens. The current findings suggested that a well-developed oviduct, particularly a magnum with considerable weight and length, is essential for proper egg formation and shell development.

CONCLUSION

The Boschveld chicken breed exhibited distinctive characteristics in the weights and numbers of small white, large white, and small yellow follicles. Furthermore, the current findings indicated substantial variance among the weights of pre-ovulatory follicles. It is concluded that the magnum was the longest and heaviest segment of the oviduct, with Follicle 1 ovarian follicle being the heaviest pre-ovulatory follicle. It is recommended to implement strategies that enhance follicle development and improve oviduct function to potentially increase egg production rates in Boschveld chickens. Additionally, conducting further research on reproductive organ morphology across different chicken breeds could provide valuable insights into their reproductive biology.

DECLARATIONS

Availability of data and materials

Data supporting the study's findings are available upon reasonable request from the corresponding author.

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Authors' contributions

Tyasi Thobela Louis conducted the conceptualisation, original design, resource provision, and supervision, also contributing to data processing, interpretation, writing, and manuscript revision. Mothiba Mmolao Leah contributed by providing images, collecting samples, processing and interpreting data, as well as writing and revising the manuscript. Hloko Victoria Rankoatse contributed to the project by providing images, collecting samples, interpreting data, writing,

and revising the manuscript. Sathekge Lindiwe Johannah contributed to data interpretation, writing, and revising the manuscript. All authors checked and approved the final edition of the manuscript.

Ethical considerations

All authors have reviewed and confirmed the originality and final version of the manuscript. The study adhered to ethical standards, ensuring no instances of plagiarism, data fabrication, or redundant publication.

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Competing interests

The authors disclosed no potential conflicts of interest.

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